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Alternate transcripts of the *Drosophila* "activator" E2F are necessary for maintenance of cell cycle exit during development

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ABSTRACT

The E2F family of transcription factors are evolutionarily conserved regulators of the cell cycle that can be divided into two groups based on their ability to either activate or repress transcription. In *Drosophila*, there is only one "activator" E2F, dE2F1, which provides all of the pro-proliferative activity of E2F during development. Interestingly, the *de2f1* gene can be transcribed from multiple promoters resulting in six alternate transcripts. In this study, we sought to investigate the biological significance of the alternate transcriptional start sites. We focused on the *de2f1* promoter region where tissue and cell-type specific enhancer activities were observed at the larval stage. While a genomic deletion of this region, $de2f1^{\Delta RA}$, decreased the overall expression level of dE2F1, flies developed normally with no obvious proliferation defects. However, a detailed analysis of the $de2f1^{\Delta RA}$ mutant eye imaginal discs revealed that dE2F1 is needed for proper cell cycle exit. We discovered that dE2F1 expression during G1 arrest prior to the differentiation process of the developing eye is important for maintaining cell cycle arrest at a later stage of the eye development. Overall, our study suggests that specific alternate transcripts of "activator" E2F, dE2F1, may have a dual function on cell cycle progression and cannot simply be viewed as a pro-proliferative transcription factor.

1. Introduction

The first E2F family transcription factor was identified in a biochemical assay as a cellular factor that binds to the Early E2 region of the adenovirus genome (Yee et al., 1989). Since its discovery, families of E2F transcription factors have been identified from nematodes to mammals (van den Heuvel and Dyson, 2008). To date, the best-characterized function of E2F transcription factors is their ability to regulate cell cycle progression (Dyson, 1998; Muller and Helin, 2000). E2Fs heterodimerize with Dimerization Partner, DP, to regulate many cell cycle genes, particularly those involved in S-phase. In turn, E2F activity is governed by a physical interaction with Rb family proteins, preventing inappropriate transcription of E2F target genes. Rb binding to E2F is periodically inactivated by cyclin-dependent kinases (CDKs), allowing E2F to activate transcription. This mechanism of coupling E2F-dependent transcription to the cell cycle is highly conserved among different species. Importantly, while E2F's regulation during the cell cycle has been extensively studied, little is known regarding E2F regulation in the context of animal development.

E2F family proteins are classified as either "activator" or "repressor" E2Fs depending on their effect on transcription (Stevaux and Dyson, 2002; Trimarchi and Lees, 2002). In mammals, E2F1, E2F2 and E2F3 are considered to be "activator" E2Fs, although E2F3b, which is an E2F3 protein having an alternate exon 1, can act as a repressor in specific contexts (Aslanian et al., 2004; Asp et al., 2009). The "activator" E2Fs are preferentially bound and regulated by the pRb tumor suppressor protein. The remaining E2Fs, E2F4–8, are "repressor" E2Fs which are more diverse than "activator" E2Fs in their protein structure and mode of action. For example, E2F4 and E2F5 form a protein complex called DREAM (Drosophila RB, E2F, and MuvB) to repress transcription while E2F6 interacts with polycomb group proteins (Sadasivam and DeCaprio, 2013; Trimarchi et al., 2001; Ogawa et al., 2002). All E2Fs, except the uniquely mammalian E2F7 and E2F8, require DP for their activity. Importantly, because "activator" and "repressor" E2Fs bind to the same DNA motifs, they are generally thought to have antagonistic functions.

Studies on mammalian E2Fs have demonstrated a functional redundancy between family members within "activator" and "repressor" E2Fs (Gaubatz et al., 2000; Wu et al., 2001). Recently, Tsai et al. elegantly demonstrated that different "activator" E2F proteins can carry out similar functions during development given

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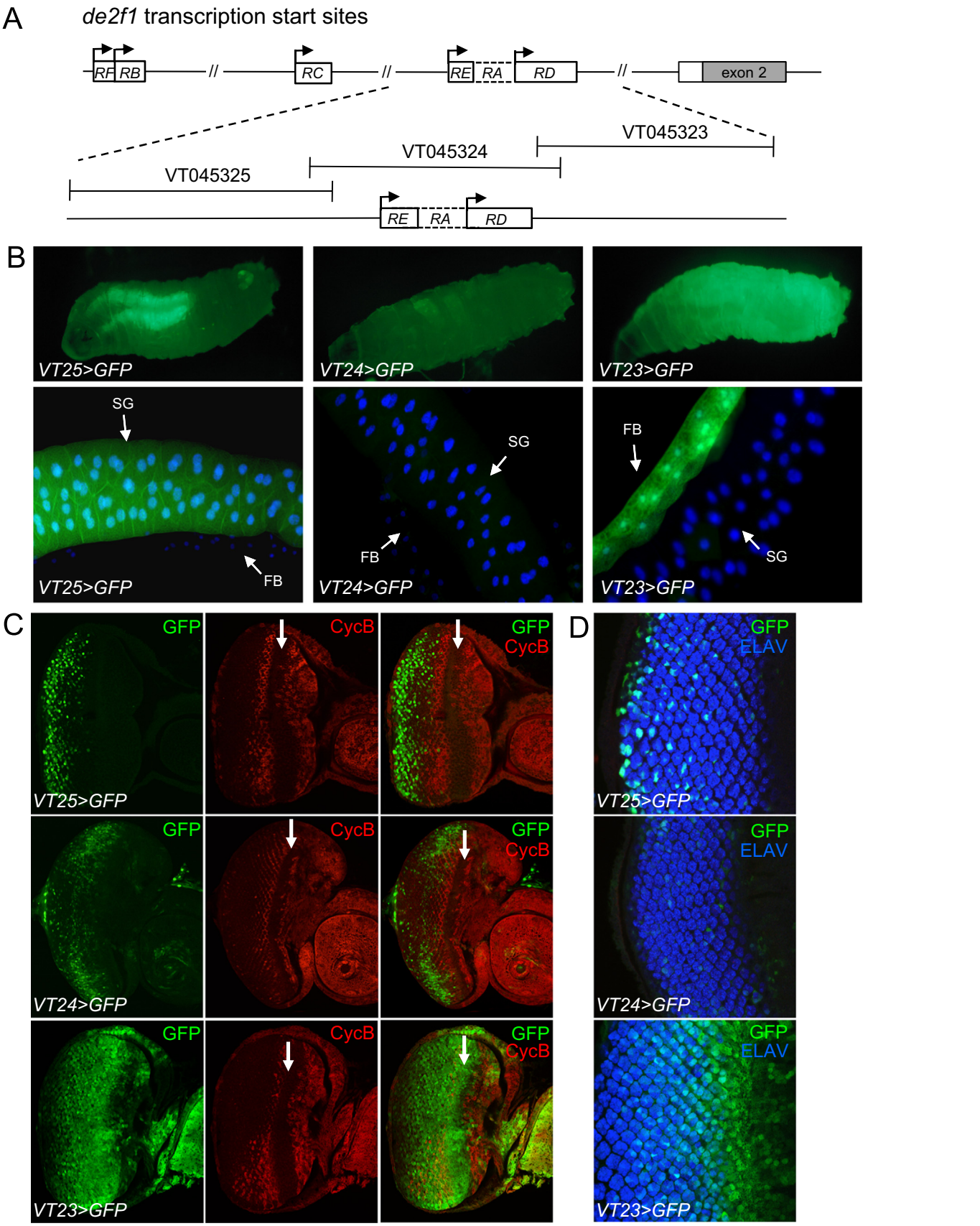

Fig. 1. The genomic region surrounding the *RA* 5′UTR contains cis-regulatory elements that are activated in specific developmental contexts. (A) A schematic of the five alternate transcription start sites of *de2f1* is shown. Black arrows indicate transcriptional start sites producing the six different transcripts named *RA, RB, RC, RD, RE,* and *RF. RA* and *RE* have the same transcription start site but are alternatively spliced to produce *de2f1* transcripts with different 5′ untranslated regions (5′UTRs). Below is an enlarged schematic of the *RA* region outlining the genomic location of the cis-regulatory elements utilized by three Vienna-Tile (VT) GAL4 lines: *VT045325 (VT25)*, *VT045324 (VT24)*, and *VT045323 (VT23)*. Note the genomic regions utilized by the VT-GAL4 lines are upstream (*VT25*), encompassing (*VT24*), and downstream (*VT23*) to the *RE/RA/RD* 5′UTRs. (B–D) A reporter analysis was performed by crossing the three VT-GAL4 lines to *UAS-GFP*. (B) GFP expression was documented in the whole larvae (top row), salivary glands (SG), and fat bodies (FB, bottom row). For salivary glands and fat bodies, DNA is visualized by DAPI staining (blue). (C) Eye discs of VT-GAL4 lines expressing *UAS-GFP* were co-stained with CycB (red) to visualize the morphogenetic furrow (marked by an arrow). (D) Higher magnification of the posterior region of the eye disc is shown co-stained with a neuronal marker ELAV (blue).